

Pectobacterium atrosepticum exopolysaccharides: Identification, molecular structure, formation under stress and in planta conditions

Gorshkov V., Islamov B., Mikshina P., Petrova O., Burygin G., Sigida E., Shashkov A., Daminova A., Ageeva M., Idiyatullin B., Salnikov V., Zuev Y., Gorshkova T., Gogolev Y.
Kazan Federal University, 420008, Kremlevskaya 18, Kazan, Russia

Abstract

© The Author 2017. Published by Oxford University Press. All rights reserved. In the present study, we identified exopolysaccharides of the harmful phytopathogenic bacterium *Pectobacterium atrosepticum* SCRI1043 and characterized the molecular structure of these polymers. The synthesis of the target polysaccharides was shown to be induced under starvation conditions. Moreover, intensive accumulation of exopolysaccharides occurred during the colonization by bacteria of the xylem vessels of infected plants, where microorganisms formed specific 3D "multicellular" structures-bacterial emboli. Thus, the identified polymers are likely to be involved in the adaptation and virulence of bacteria of *Pectobacterium* genus.

<http://dx.doi.org/10.1093/glycob/cwx069>

Keywords

bacterial emboli, exopolysaccharides, extracellular matrix, molecular structure of polysaccharides, plant-microbe interactions

References

- [1] Al Safadi R, Abu-Ali G, Sloup R, Rudrik J, Waters C, Eaton K, Manning S. 2012. Correlation between in vivo biofilm formation and virulence gene expression in *Escherichia coli* O104: H4. *PLoS One*. 7:e41628.
- [2] Arbatsky N, Wang M, Shashkov A, Feng L, Knirel Y, Wang L. 2010. Structure of the O-polysaccharide of *Cronobacter sakazakii* O1 containing 3-(N-acetyl-l-alanyl) amino-3, 6-dideoxy-d-glucose. *Carbohydr Res*. 345:2095-2098.
- [3] Armitage J, Berry R. 2010. Time for bacteria to slow down. *Cell*. 141:24-26.
- [4] Bazaka K, Crawford R, Nazarenko E, Ivanova E. 2011. Bacterial extracellular polysaccharides. In: Linke D, Goldman A, editors. *Advances in experimental medicine and biology. Bacterial adhesion* 715. Netherlands: Springer. p. 213-226.
- [5] Bell KS, Sebaihia M, Pritchard L, Holden MT, Hyman LJ, Holeva MC, Thomson NR, Bentley SD, Churcher LJ, Mungall K, et al. 2004. Genome sequence of the enterobacterial phytopathogen *Erwinia carotovora* subsp. *atroseptica* and characterization of virulence factors. *Proc Natl Acad Sci USA*. 101:11105-11110.
- [6] Bharati B, Sharma I, Kasetty S, Kumar M, Mukherjee R, Chatterji D. 2012. A full-length bifunctional protein involved in c-di-GMP turnover is required for long-term survival under nutrient starvation in *Mycobacterium smegmatis*. *Microbiology*. 158:1415-1427.
- [7] Bharati B, Swetha R, Chatterji D. 2013. Identification and characterization of starvation induced *msdgc-1* promoter involved in the c-di-GMP turnover. *Gene*. 528:99-108.

- [8] Boehm A, Kaiser M, Li H, Spangler C, Kasper C, Ackermann M, Kaefer V, Sourjik V, Roth V, Jenal U. 2010. Second messenger-mediated adjustment of bacterial swimming velocity. *Cell*. 141:107-116.
- [9] Bukharov A, Skvortsov I, Ignatov V, Shashkov A, Knirel Y, Dabrowski J. 1993. Structure of the O-specific polysaccharide of *Xanthomonas campestris* NCPPB 45 lipopolysaccharide. *Carbohydr Res*. 241:309-316.
- [10] Bylund J, Burgess L, Cescutti P, Ernst R, Speert D. 2006. Exopolysaccharides from *Burkholderia cenocepacia* inhibit neutrophil chemotaxis and scavenge reactive oxygen species. *J Biol Chem*. 281:2526-2532.
- [11] Charkowski A, Blanco C, Condemine G, Expert D, Franza T, Hayes C, Hugouvieux-Cotte-Pattat N, López Solanilla E, Low D, Moleleki L, et al. 2012. The role of secretion systems and small molecules in softrot *Enterobacteriaceae* pathogenicity. *Annu Rev Phytopathol*. 50: 425-449.
- [12] Chouly C, Colquhoun I, Jodelet A, York G, Walker G. 1995. NMR studies of succinoglycan repeating-unit octasaccharides from *Rhizobium meliloti* and *Agrobacterium radiobacter*. *Int J Biol Macromol*. 17:357-363.
- [13] Compant S, Clément C, Sessitsch A. 2010. Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. *Soil Biol Biochem*. 42: 669-678.
- [14] Davidsson P, Kariola T, Niemi O, Palva E. 2013. Pathogenicity of and plant immunity to soft rot pectobacteria. *Front Plant Sci*. 4:191.
- [15] D'Haese W, Holsters M. 2004. Surface polysaccharides enable bacteria to evade plant immunity. *Trends Microbiol*. 12:555-561.
- [16] Dubois M, Gilles K, Hamilton J, Rebers P, Smith F. 1956. Colorimetric method for determination of sugars and related substances. *Anal Chem*. 28: 350-356.
- [17] Fedonenko Y, Burygin G, Popova I, Sigida E, Surkina A, Zdrovenko E, Konnova S. 2013. Immunochemical characterization of the capsular polysaccharide of *Azospirillum irakense* KBC1. *Curr Microbiol*. 67: 234-239.
- [18] Fedonenko Y, Konnova O, Zatonsky G, Shashkov A, Konnova S, Zdrovenko E, Ignatov VV, Knirel YA. 2004. Structure of the O-polysaccharide of the lipopolysaccharide of *Azospirillum irakense* KBC1. *Carbohydr Res*. 339:1813-1816.
- [19] Fry S. 1988. The growing plant cell wall: Chemical and metabolic analysis (monographs and surveys in the biosciences). Harlow, UK: Longman Scientific & Technical.
- [20] Genin S. 2010. Molecular traits controlling host range and adaptation to plants in *Ralstonia solanacearum*. *New Phytol*. 187:920-928.
- [21] Gidley M, Dea I, Eggleston G, Morris E. 1987. Structure and gelation of *Rhizobium* capsular polysaccharide. *Carbohydr Res*. 160:381-396.
- [22] Gorshkov V, Daminova A, Ageeva M, Petrova O, Gogoleva N, Tarasova N, Gogolev Y. 2014. Dissociation of a population of *Pectobacterium atrosepticum* SCRI1043 in tobacco plants: Formation of bacterial emboli and dormant cells. *Protoplasma*. 251:499-510.
- [23] Gorshkov V, Daminova A, Mikshina P, Petrova O, Ageeva M, Salnikov V, Gorshkova TA, Gogolev YV. 2016. Pathogen-induced conditioning of the primary xylem vessels-a prerequisite for the formation of bacterial emboli by *Pectobacterium atrosepticum*. *Plant Biol*. 18:609-617.
- [24] Gorshkov V, Petrova O, Gogoleva N, Gogolev Y. 2010. Cell-to-cell communication in the populations of enterobacterium *Erwinia carotovora* ssp. *atroseptica* SCRI1043 during adaptation to stress conditions. *FEMS Immunol Med Microbiol*. 59:378-385.
- [25] Gorshkov V, Petrova O, Mukhametshina N, Ageeva M, Mulyukin A, Gogolev Y. 2009. Formation of "non-culturable" dormant forms of the phytopathogenic enterobacterium *Erwinia carotovora*. *Microbiologiya (Moscow)*. 78:585-592.
- [26] Hainfeld J, Powell R. 2000. New frontiers in gold labeling. *J Histochem Cytochem*. 48:471-480.
- [27] Hitchcock P, Brown T. 1983. Morphological heterogeneity among *Salmonella* lipopolysaccharide chemotypes in silver-stained polyacrylamide gels. *J Bacteriol*. 154:269-277.
- [28] Iyer A, Mody K, Jha B. 2005. Biosorption of heavy metals by a marine bacterium. *Mar Pollut Bull*. 50:340-343.
- [29] Janczarek M, Rachwa K, Ciesla J, Ginalska G, Bieganski A. 2015. Production of exopolysaccharide by *Rhizobium leguminosarum* bv. *trifolii* and its role in bacterial attachment and surface properties. *Plant Soil*. 388:211-227.
- [30] Jansson P, Lindberg B, Ljunggren H. 1979. Structural studies of the *Rhizobium trifolii* extracellular polysaccharide. *Carbohydr Res*. 75: 207-220.
- [31] Karkhanis YD, Zeltner JY, Jackson JJ, Carlo D. 1978. A new and improved microassay to determine 2-keto-3-deoxyoctonate in lipopolysaccharide of Gram-negative bacteria. *Anal Biochem*. 85:595-601.
- [32] Koczan J, McGrath M, Zhao Y, Sundin G. 2009. Contribution of *Erwinia amylovora* exopolysaccharides amylovan and levan to biofilm formation: Implications in pathogenicity. *Phytopathology*. 99:1237-1244.
- [33] Koutsoudis M, Tsaltas D, Minogue T, von Bodman S. 2006. Quorum-sensing regulation governs bacterial adhesion, biofilm development, and host colonization in *Pantoea stewartii* subspecies *stewartii*. *Proc Natl Acad Sci USA*. 103:5983-5988.

- [34] Kubheka G, Coutinho T, Moleleki N, Moleleki L. 2013. Colonization patterns of an mCherry-tagged *Pectobacterium carotovorum* subsp. *brasiliense* strain in potato plants. *Phytopathology*. 103:1268-1279.
- [35] Lehman A, Long S. 2013. Exopolysaccharides from *Sinorhizobium meliloti* can protect against H₂O₂-dependent damage. *J Bacteriol*. 195:5362-5369.
- [36] Leigh J, Coplin D. 1992. Exopolysaccharides in plant-bacterial interactions. *Annu Rev Microbiol*. 46:307-346.
- [37] Limoli D, Jones C, Wozniak D. 2015. Bacterial extracellular polysaccharides in biofilm formation and function. *Microbiol Spec*. 3, doi:10. 1128/ microbiolspec. MB-0011-2014.
- [38] Mayer H, Tharanathan R, Weckesser J. 1985. Analysis of lipopolysaccharides of gram-negative bacteria. *Meth Microbiol*. 18:157-207.
- [39] Moleleki L, Pretorius R, Tanui C, Mosina G, Theron J. 2017. A quorum sensing defective mutant of *Pectobacterium carotovorum* ssp. *brasiliense* 1692 is attenuated in virulence and unable to occlude xylem tissue of susceptible potato plant stems. *Mol Plant Pathol*. 18:32-44.
- [40] Molinaro A, Silipo A, Lanzetta R, Newman M, Dow J, Parrilli M. 2003. Structural elucidation of the O-chain of the lipopolysaccharide from *Xanthomonas campestris* strain 8004. *Carbohydr Res*. 338:277-281.
- [41] Murashige T, Skoog F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol Plantarum*. 15:473-497.
- [42] Nichols C, Guezennec J, Bowman J. 2005. Bacterial exopolysaccharides from extreme marine environments with special consideration of the southern ocean, sea ice, and deep-sea hydrothermal vents: A review. *Mar Biotechnol*. 7:253-271.
- [43] Nimtz M, Mort A, Domke T, Wray V, Zhang Y, Qiu F, Coplin D, Geider K. 1996. Structure of amylovoran, the capsular exopolysaccharide from the fire blight pathogen *Erwinia amylovora*. *Carbohydr Res*. 287:59-76.
- [44] Perez-Mendoza D, Coulthurst S, Sanjuán J, Salmond G. 2011. N-Acetylglucosamine-dependent biofilm formation in *Pectobacterium atrosepticum* is cryptic and activated by elevated c-di-GMP levels. *Microbiology*. 157:3340-3348.
- [45] Perombelon M. 2002. Potato diseases caused by soft rot erwinias: An overview of pathogenesis. *Plant Pathol*. 51:1-12.
- [46] Petrova O, Gorshkov V, Daminova A, Ageeva M, Moleleki L, Gogolev Y. 2014. Stress response in *Pectobacterium atrosepticum* SCRI1043 under starvation conditions: Adaptive reactions at a low population density. *Res Microbiol*. 165:119-127.
- [47] Petrova O, Gorshkov V, Sergeeva I, Daminova A, Ageeva M, Gogolev Y. 2016. Alternative scenarios of starvation-induced adaptation in *Pectobacterium atrosepticum*. *Res Microbiol*. 167:254-261.
- [48] Qurashi A, Sabri A. 2012. Bacterial exopolysaccharide and biofilm formation stimulate chickpea growth and soil aggregation under salt stress. *Braz J Microbiol*. 43:1183-1191.
- [49] Ray T, Smith A, Wait R, Hignett R. 1987. Structure of the sidechain of lipopolysaccharide from *Erwinia amylovora* T. *Eur J Biochem*. 170:357-361.
- [50] Reinhold B, Chan S, Reuber T, Marra A, Walker G, Reinhold V. 1994. Detailed structural characterization of succinoglycan, the major exopolysaccharide of *Rhizobium meliloti* Rm1021. *J Bacteriol*. 176:1997-2002.
- [51] Reuber T, Walker G. 1993. The acetyl substituent of succinoglycan is not necessary for alfalfa nodule invasion by *Rhizobium meliloti* Rm1021. *J Bacteriol*. 175:3653-3655.
- [52] Reynolds E. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J Cell Biol*. 17:208-212.
- [53] Roper M. 2011. *Pantoea stewartii* subsp. *stewartii*: Lessons learned from xylem-dwelling pathogen of sweet corn. *Mol Plant Pathol*. 12: 628-637.
- [54] Sambrook J, Fritsch E, Maniatis T. 1989. *Molecular cloning: A laboratory manual*, 2nd ed. Harbor, NY: Cold Spring Harbor Laboratory Press.
- [55] Senchenkova S, Shashkov A, Knirel Y, Ahmed M, Mavridis A, Rudolph K. 2005. Structure of the O-polysaccharide of *Erwinia carotovora* ssp. *atroseptica* GSPB 9205 containing a new higher branched monosaccharide. *Russ Chem B*. 54:1276-1281.
- [56] Skorupska A, Janczarek M, Marczak M, Mazur A, Król J. 2006. Rhizobial exopolysaccharides: Genetic control and symbiotic functions. *Microb Cell Fact*. 5:7.
- [57] Smol'kina ON, Kachala VV, Fedonenko YP, Burygin GL, Zdorovenko EL, Matora LY, Konnova SA, Ignatov VV. 2010. Capsular polysaccharide of the bacterium *Azospirillum lipoferum* Sp59b: Structure and antigenic specificity. *Biochemistry (Moscow)*. 75:606-613.
- [58] Singh N, Asthana R, Kayastha A, Pandey S, Chaudhary A, Singh S. 1999. Thiol and exopolysaccharide production in a cyanobacterium under heavy metal stress. *Process Biochem*. 35:63-68.
- [59] Snyder D, Gibson D, Heiss C, Kay W, Azadi P. 2006. Structure of a capsular polysaccharide isolated from *Salmonella enteritidis*. *Carbohydr Res*. 341: 2388-2397.
- [60] Stankowski J, Mueller B, Zeller S. 1993. Location of a second O-acetyl group in xanthan gum by the reductive-cleavage method. *Carbohydr Res*. 241: 321-326.

- [61] Starr KF, Porsch EA, Heiss C, Black I, Azadi P, Geme J III. 2013. Characterization of the *Kingella kingae* polysaccharide capsule and exopolysaccharide. *PLoS One*. 8:e75409.
- [62] Tarasova N, Gorshkov V, Petrova O, Gogolev Y. 2013. Potato signal molecules that activate pectate lyase synthesis in *Pectobacterium atrosepticum*SCRI1043. *World J Microbiol Biotechnol*. 29:1189-1196.
- [63] Torres LG, Brito E, Galindo E, Choplin L. 1993. Viscous behaviour of xanthan aqueous solutions from a variant strain of *Xanthomonas campestris*. *J Ferment Bioeng*. 75:58-64.
- [64] Tsai C, Frasch C. 1982. A sensitive silver stain for detecting lipopolysaccharides in polyacrylamide gels. *Anal Biochem*. 119:115-119.
- [65] Videira P, Fialho A, Geremia R, Breton C, Isabel S. 2001. Biochemical characterization of the 1, 4-glucuronosyltransferase GelK in the gellan gumproducing strain *Sphingomonas paucimobilis* ATCC 31461. *Biochem J*. 358:457-464.
- [66] Vu B, Chen M, Crawford R, Ivanova E. 2009. Bacterial extracellular polysaccharides involved in biofilm formation. *Molecules*. 14:2535-2554.
- [67] Wang H, Jiang X, Mu H, Liang X, Guan H. 2007. Structure and protective effect of exopolysaccharide from *P. agglomerans* strain KFS-9 against UV radiation. *Microbiol Res*. 162:124-129.
- [68] Westphal O, Jann K. 1965. Bacterial lipopolysaccharides: Extraction with phenol-water and further applications of the procedure. In: Whistler RL, Wolfrom ML, editors. *Methods in carbohydrate chemistry*. New York: Academic press. p. 83-91.
- [69] Yamazaki M, Thorne L, Mikolajczak M, Armentrout R, Pollok T. 1996. Linkage of genes essential for synthesis of a polysaccharide capsule in *Sphingomonas* strain S88. *J Bacteriol*. 178:2676-2687.
- [70] Yang B, Ding Q, Montgomery R. 2002. Extracellular polysaccharides of *Erwinia furlu*, a bacterium associated with a fungal canker disease of *Eucalyptus* spp. *Carbohydr Res*. 337:2469-2480.
- [71] Yang H, Deng J, Yuan Y, Fan D, Zhang Y, Zhang R, Han B. 2015. Two novel exopolysaccharides from *Bacillus amyloliquefaciens* C-1: Antioxidation and effect on oxidative stress. *Curr Microbiol*. 70: 298-306.
- [72] Zegans M, Wozniak D, Griffin E, Toutain-Kidd C, Hammond J, Garfoot A, Lam J. 2012. *Pseudomonas aeruginosa* exopolysaccharide Psl promotes resistance to the biofilm inhibitor polysorbate 80. *Antimicrob Agents Chemother*. 56:4112-4122.